Enemies and Turncoats: Bovine tuberculosis exposes pathogenic potential of Rift Valley fever virus in a common host, African Buffalo (Syncerus caffer)

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Enemies and Turncoats: Bovine tuberculosis exposes pathogenic potential of Rift Valley fever virus in a common host, African Buffalo (*Syncerus caffer)*

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Abstract

The ubiquity and importance of parasite co-infections in populations of free-living animals is beginning to be recognized, but few studies have demonstrated differential fitness effects of single infection versus co-infection in free-living populations. We investigated interactions between the emerging bacterial disease bovine tuberculosis (BTB) and the previously existing viral disease Rift Valley fever (RVF) in a competent reservoir host, African buffalo, combining data from a natural outbreak of RVF in captive buffalo at a buffalo breeding facility in 2008 with data collected from a neighboring free-living herd of African buffalo in Kruger National Park. RVF infection was twice as likely in individual BTB + buffalo as in BTB- buffalo, which, according to a mathematical model, may increase RVF outbreak size at the population level. In addition, coinfection was associated with a far higher rate of fetal abortion than other infection states. Immune interactions between BTB and RVF may underlie both of these interactions, since animals with BTB had decreased innate immunity and increased pro-inflammatory immune responses. This study is one of the first to demonstrate how the consequences of emerging infections extend beyond direct effects on host health, potentially altering the dynamics and fitness effects of infectious diseases that had previously existed in the ecosystem on free-ranging wildlife populations.

Introduction:

Anthropogenic changes to the environment - such as shifts in biotic assemblages, altered climate patterns, and reduced environmental predictability - have led to alterations in disease patterns worldwide [1, 62]]. These altered patterns include the emergence of new pathogens and parasites, often via spillover from one species to another, and pre-existing pathogens and parasites increasing in geographic range [1]. These changing infection patterns can cause cascading effects through the host population, such as population declines [2] due to increased mortality as seen in rinderpest outbreaks in sub-Saharan Africa [3] or decreased fecundity as experienced by koala bears infected with chlamydia [4]. Not only do infectious diseases have direct effects on host populations, but they may also alter the spread and fitness effects of other pathogens within the host population due to mechanisms such as change in susceptibility to infection, increased mortality [5], or decreased fecundity [6] of coinfected individuals, thereby altering established host-parasite dynamics.

Recent literature has shown that a native pathogen community may alter the success of an invading infectious disease [7, 56] . For instance, European eels with higher micro-parasite and macro-parasite richness were more likely to be infected by the invading parasite, *Anguillicoloides crassus* [8]. A two-parasite disease model showed that native nematodes might facilitate the invasion of bovine tuberculosis (BTB) in African buffalo [9]. However, very little work has investigated how the presence of an emerging pathogen may alter the dynamics of previously existing native infections. For the purposes of this paper we define an emerging disease as the World Health Organization does, "a disease that has appeared in the population for the first time or that might have previously existed but is rapidly increasing in incidence or geographic range". We use the term native disease to mean a disease that existed in the ecosystem and host species prior to the emerging disease.

Emerging and native parasites can interact via the host immune system [14]. An emerging pathogen may erode host defenses against native infections, increasing transmission risk of the native infection in infected individuals [10]. Alternatively, the emerging infection may remove susceptible animals from the pool by cross-protective immune response [11], changes in host behavior [12] or mortality [13], reducing the transmission opportunities for native infections. If the immune response mounted to one parasite is cross-protective to another, then infection with one parasite can prevent the other from establishing. In contrast, immune responses may be mutually antagonistic [14, 15]. An immune response to one type of parasite may allow infection of another by preventing an appropriate immune response [16, 17], creating a facilitative effect.

We studied an outbreak of Rift Valley fever (RVF), a native pathogen, in African buffalo (*Syncerus caffer)* infected with *Mycobacterium bovis* (causal agent of BTB), which is an emerging disease in the area of study, Kruger National Park (KNP). We investigated whether animals with BTB have differential risk of acquiring RVF, and compared the fitness effects of co-infection with single infections. We hypothesized that interactions between *M. bovis* and Rift Valley fever virus may be mediated via their effects on, and responses to, host immunity.

BTB is not native to sub-Saharan Africa and is considered to be an emerging infection in African wildlife [57]. BTB emerged into the landscape in either the 1960's [58] or 1980's [37] and was first detected in the 1990's [59] in Kruger National Park. Since that time BTB has been spreading northward in the park with prevalence increasing over time throughout the park [19] and just recently crossing the northern boundary of the park into Zimbabwe [60]. BTB in African buffalo is an excellent system to study immune mediated interactions between parasites because BTB has moderate effects on the survival of African buffalo [19,63], but modifies the

host immune system to ensure its survival within the host for the lifetime of the buffalo [20,21]. For instance, there is evidence that cattle with BTB have a suppressed innate immune response [22]. In addition to an altered innate immune response, BTB affects the cell-mediated acquired immune system, with an increase in inflammatory cytokines (Th1 skew) that is linked to increased pathology associated with BTB infection [23].

RVF is a mosquito-transmitted intracellular viral disease with numerous mammalian hosts, including African buffalo. RVF is considered native to South Africa, having existed in the ecosystem prior to bovine tuberculosis and been identified as a spillover infection from animals to people in 1952 (24). Outbreaks are known to occur in domestic animals every 5 to 7 years during the wet season [24], but the virus may cycle undetected in wildlife populations during the interepidemic period [27,61]. It has mild effects on African buffalo, primarily causing a short-term illness that passes within 2-3 days - much like a seasonal cold in humans - with severe effects primarily limited to occasional abortion [24]. The ability of hosts to resist RVF infection is dependent on a strong innate immune response [25]. Since BTB can suppress the innate immune response, we hypothesize that animals previously infected with BTB may be more susceptible to infection with RVF.

We investigated the role of bovine tuberculosis in a natural RVF outbreak in a captive population of African buffalo at a breeding facility, and an adjacent free-ranging population in Kruger National Park. We analyzed data from the captive population to determine whether animals with BTB were more or less likely to become infected with RVF during the outbreak and to suffer fitness consequences in the form of abortions. We then tested whether patterns found in the captive population were mirrored in the free-ranging buffalo population. To investigate potential mechanisms mediating epidemiological patterns we investigated whether

buffalo with BTB have an altered immune response that may affect the likelihood of acquiring RVF or suffering abortion, during an outbreak. Finally, we used a mathematical model to determine how observed changes in individual susceptibility could scale up to alter population level patterns of RVF transmission.

Methods:

COINFECTION PATTERNS

RVF Outbreak in the captive population

In 2008 an outbreak of RVF occurred in and around Kruger National Park [26]. We collected data on the captive animals from a buffalo breeding facility on the southern boundary of Kruger National Park, the Nkomazi area, on RVF infection prior to, during, and post outbreak. During the year prior to the outbreak (2007) the buffalo breeding facility was free of RVF. The outbreak, first noted in the facility on January 14, 2008, was contained by the end of February when the entire herd was vaccinated for RVF. Prior to vaccination, but after the outbreak, blood was collected from each individual and was serologically tested for RVF using a hemagglutination-inhibition (HAI) titration assay at Onderstepoort Veterinary Institute in Pretoria, South Africa [27,28]. The breeding facility had both BTB + buffalo and BTB - buffalo, but animals were known to be brucellosis free, were on a deworming schedule to prevent gastrointestinal helminth infection, and were regularly treated with antiparasitic dips to reduce ticks and tick-borne infections. Animals were assigned BTB status based on the results of multiple caudal fold skin tests prior to the outbreak; all had been tested at least once in the prior year. This assay is described in the OIE terrestrial manual (2012) and has been used in African buffalo [29,30]. Briefly, animals are intradermally injected with bovine tuberculin and the

swelling response is measured 72 hours later with a swelling response greater than 2 mm considered positive. BTB - buffalo were certified disease-free based on the results of 2 prior BTB tests. The sensitivity and specificity of caudal fold skin BTB tests is respectively, 80-91% and 95-100% in cattle [31-33]; 80.9% and 90.2% in African buffalo (JP Raath, unpublished data). BTB +and BTB - buffalo were maintained in separate, but similar bomas (enclosures approximately 0.25 km apart), and had no direct contact with one another. While these bomas did not allow direct contact they were close enough for infected vectors to fly from one to the other - although whether they did is not a variable we assessed.

To determine the cause of mortality in the juvenile and adult buffalo during the outbreak state veterinarians performed full necropsies and noted the presence of lesions concordant with Rift Valley fever infection [24]. Infection was confirmed with immunohistochemical staining [34]. Aborted fetuses were also collected and necropsies and immunohistochemistry were once again used for confirmation of RVF infection. Additional RVF confirmatory tests on fetuses were performed using RT-PCR [35] of fetal blood samples. All immunohistochemistry and PCR analyses were conducted at the Onderstepoort Veterinary Institute.

To assess whether abortion rates were different in coinfected and singly infected individuals we first determined what proportion of individuals should have been pregnant on the buffalo breeding facility prior to the outbreak. Previous non-outbreak years pregnancy and birthing data were used to determine an interbirth interval on the buffalo breeding facility of 462 days (from 1999-2007, n=756) and an average pregnancy rate of 73% in adult female cows, which did not differ between BTB+ and BTB- buffalo. When calculating abortion rates in the captive population we used a denominator of 73% of the total reproductive females. We then assessed whether abortion rates were different between the 4 disease groups (coinfected, single

RVF infection, single BTB infection, uninfected) using a non-parametric ANOVA - Kruskal Wallis with Dunn multiple comparisons.

RVF outbreak in the free-ranging population

To evaluate whether BTB/RVF coinfection patterns found in the buffalo breeding facility were mirrored in a free living population we sampled 96 free-living young female buffalo in the southern portion of Kruger National Park (where BTB prevalence is approximately 50% [63, 64] near the buffalo breeding facility in October 2008 (approximately 7 months after the outbreak of RVF) as part of a larger disease study [63]. Animals were chemically immobilized with etorphine hydrochloride, azaperone and ketamine by darting from a helicopter. After immobiliization, age, body condition and pregnancy status were determined. Animal ages were assessed from incisor emergence patterns for buffalo 2–5 years old and from tooth wear of the first incisor for buffalo 6 years of age and older [36]. Body condition was measured by visually inspecting and palpating four areas on the animal where fat is stored in buffalo: ribs, spine, hips and base of tail. Each area was scored from 1 (very poor) to 5 (excellent) and a body condition score calculated as the average of all four areas [37]. This index is correlated with the kidney fat index [38]. Pregnancy status was assessed by rectal palpation [30,36,42], performed by an experience wildlife veterinarian. Blood was collected by jugular venipuncture into lithium heparinized tubes (for BTB diagnostics) and tubes with no additive (RVF diagnostics) and transported back the lab on ice within 8 hours of collection. Feces was collected rectally and transported back to the laboratory on ice for fecal egg counts of strongyle nematodes and coccidia (for specific methods see [42]). Following data collection, immobilization was reversed using M5050 (diprenorphine). Animals were chemically restrained for no longer than 60

minutes. Time of capture and duration of anesthesia were initially included in all statistical models but were never found to be important predictors.

We determined RVF serostatus with the virus neutralization test, which has a sensitivity and specificity of nearly 100% [39] and can be used to look for antibodies in serum. Tuberculosis infection status was determined using a standard whole-blood gamma interferon assay protocol (BOVIGAM) [40]. In brief, this assay is performed by comparing the *in vitro* IFNg response to *Mycobacterium bovis* antigen (bovine tuberculin) to the IFNg response to an avian tuberculin antigen and background IFNg levels in the absence of antigenic stimulation. This assay has been optimized for use in African buffalo [41], and blood cells from buffalo infected with *M. bovis* show a pronounced spike in IFNg production in response to bovine but not avian tuberculin, whereas bovine tuberculin challenge does not induce IFNg production in the blood of unexposed animals [41]. We implemented the gamma interferon assay with the BOVIGAM enzyme-linked immunosorbent assay kit (Prionics), which has a sensitivity of 86% and a specificity of 92% in African buffalo [41]. We used the BOVIGAM test instead of the skin test used at the buffalo breeding facility because the skin test was impractical in our field setting; the skin test requires 2 captures in 3 days whereas the BOVIGAM test can be performed on whole blood collected in 1 capture.

We performed a Fishers exact test to determine whether animals with BTB were more likely to be seropositive for RVF than their BTB - counterparts in the free-ranging population. The majority of these RVF positive animals likely converted in the 2008 outbreak: most animals were between 2-5 years old, whereas the most recent identified RVF outbreak in the area, prior to 2008, occurred in 1999 before these animals were born. We calculated a RVF prevalence ratio with and without BTB (i.e. prevalence ratio = prevalence in BTB+ buffalo / prevalence in BTB-

buffalo). To further evaluate the correlation between BTB and RVF we performed a generalized linear model with binomial distribution to evaluate whether BTB status predicted RVF status, after accounting for buffalo age, body condition, pregnancy, fecal egg count of GI nematodes and coccidia in the free-ranging population. We were unable to assess whether coinfected animals in the free-ranging population were more likely to abort than singly infected individuals, as we demonstrated in the population at the buffalo breeding facility, for two reasons. First, the population of buffalo sampled was primarily pre - reproductive (<4 years of age), and second, sampling did not exactly coincide with the RVF outbreak, and it is likely that any animal that did abort due to RVF during the outbreak was pregnant again at the time our sampling took place (~ 7 months later).

IMMUNE MECHANISMS

The 96 free-living individuals described in the methods above were followed subsequently for 4 years. Each individual was marked with a radio-collar and recaptured every 6 months (2008-2012). Any animal that died during the study period was replaced by a similarly aged animal to maintain a constant sample size of approximately 100 individuals at each recapture. At each capture period the same data were collected including age, body condition and BTB status as described above. We also collected information on a pro-inflammatory cytokine (IL12) and general innate immune capability as measured by the bactericidal assay on subsets of these animals as described below.

Bactericidal Assay

We performed the bactericidal assay as a measure of innate immune capability. The assay measures the proportion of bacteria (*E. coli*, in this case) killed by whole blood during a 30 minute period of interaction between blood and bacterial broth. Killing mechanisms include protein-mediated killing (e.g. complement, acute-phase proteins) and cell-mediated killing (e.g. phagocytosis by macrophages, neutrophils). This assay was performed as described in [42] with replicate plates between July 2010 and July 2011, for 97 individual buffalo, some of which were the same individuals as reported above for the RVF outbreak in the free-living population (n=34) and some of which were added to the study after the outbreak (n=63). Briefly, for experimental tubes whole blood and bacteria were mixed together and incubated for 30 minutes. For control tubes the same quantity of bacteria and phosphate buffered saline (PBS) were mixed. After 30 minutes the mixture was plated onto agar and the bacteria allowed to grow at 37C for 12 hours. After 12 hours the number of bacteria colonies on each plate was counted. The number of colonies killed by whole blood was determined by subtracting the number of colonies on the experimental plate from the control plate. This was used as the independent variable in statistical analyses, and we account for day-to-day variation in growth by including the number of colonies on the control plates as an offset term in all statistical models (42). A generalized linear model (quasipoisson distribution, log link) was used to determine if the number of colonies killed by whole blood differed by BTB status, body condition, age or any two-way interaction effects.

IL12 Assay

Cytokines are immunologically active proteins that aid in cell signaling during a host immune response and have been proposed as an excellent way to simplistically and realistically describe the immune profiles for the purpose of understanding within-host parasite interactions

[43]. IL12 is known to be important in immune defense against viruses and is a key pro-inflammatory cytokine [53]. We assessed IL12 production in whole blood in response to *in vitro* stimulation with two mitogens, pokeweed and live Rift Valley fever virus. Pokeweed is a general immune stimulant that is often used to induce cytokine and cell proliferation; the strain of Rift Valley fever we used was a modified live strain used in vaccines (Smithburn strain). After return from the field whole blood in lithium heparinized tubes was pipetted into 1.5 ml aliquots. Into each aliquot we added 50 ul of mitogen (30,000 live RVF virus units from Onderstepoort Biologicals or 15ug of pokeweed (Sigma L9379, rehydrated in PBS)) into experimental tubes and 50ul of PBS into control tubes. Whole blood and mitogen (PBS for controls) were incubated at 37C for 24 hours. After 24 hours the plasma was pipetted off the top of the tube, placed in cryovials and stored at -20C until analysis. The quantity of IL12 in each sample was measured using a sandwich ELISA following established protocols [44] using a commercially available antibodies designed for bovines (Abd Serotec, #MCA1782EL & MCA2173B) and recombinant bovine IL12 for the standard curve (Kingfisher, RP0077B). All samples were performed in duplicate on a 96 well plate and the mean optical density was calculated for each set of duplicate wells at a wavelength of 405nm. The mean OD was calculated for each set of duplicate wells with an average variation between wells of 5.76%. Sample concentrations were calculated using a linear standard curve and are expressed as pg/ml. The difference in IL12 detected between control and experimental tubes was used as the dependent variable in statistical analyses.

To assess if animals with BTB differed from those without BTB in IL12 production after stimulation with the nonspecific mitogen (pokeweed) we performed a generalized linear mixed model (Gaussian family, log link, dependent variable was log of the difference between IL12 in

the stimulated samples and IL12 in the nonstimulated sample) on 118 individual buffalo captured between June 2008 and August 2010 that we had repeated IL12 measurements on for a total of 419 IL12 data points. The random effects in the model were the number of plate the sample was run on and buffalo individual to avoid pseudo replication of repeated measures on the same individual. We evaluated the fixed effects including all two-way interactions of age, year of capture, BTB status, animal body condition and the amount of IL12 already in the blood before stimulation (circulating IL12). We found no significant two-way interactions and so presented the main effects model.

We then evaluated IL12 production in response to Rift valley fever virus (Smithburn strain) in a subset of 27 animals captured in September/October 2011. We calculated a proportional change in IL12 production [(IL12 in tubes with mitogen-IL12 in control tubes)/IL12 in control tubes] and assessed whether BTB + individuals also had higher IL12 response to RVF than BTB- individuals using a 2-tailed t-test on arcsine-square root transformed data.

MATHEMATICAL MODEL

We can infer, from the collected data, an individual buffalo's differential risk for contracting RVF during the outbreak based on their BTB status. However, we hypothesize that the presence of BTB not only increases the risk of RVF infection in BTB+ buffalo but in the whole herd, and that the presence of BTB could change the dynamics of RVF at the population level. To test this hypothesis, we modified a mathematical model of RVF transmission in free-living buffalo [45] to explore how the altered risk of RVF infection in BTB infected individuals may change epidemic dynamics of RVF (see Appendix 1 for details). We tested sensitivity of

the model output to the proportion of the herd infected with BTB and to the magnitude of change in susceptibility to RVF for BTB+ buffalo.

We altered the model to account for BTB presence by dividing the herd into BTB + and BTB - groups, changing one parameter in the model to account for increased susceptibility of BTB + buffalo to RVF infection via infected mosquito bite. Since we have no evidence for a difference in buffalo-to-mosquito transmission probability, we leave the probability of a susceptible mosquito acquiring the virus after biting an infectious buffalo unchanged. The available data is for a single RVF outbreak, so we modeled RVF spread in one rainy season, and did not explicitly include BTB transmission dynamics or buffalo population dynamics in the model. This simple and interpretable model provides a framework with which to assess the population level effects of BTB on a single RVF outbreak in the herd.

Results:

DESCRIPTIVE STATISTICS OF RVF OUTBREAK IN CAPTIVE AND FREE-LIVING BUFFALO

Two hundred and thirty five captive buffalo were tested for RVF before and during the 2008 outbreak. Of these, 60 were calves under 1 year of age, 156 were adult cows and the remaining 19 were adult bulls. There were 82 new cases of RVF recorded during the 2008 outbreak at the breeding facility, i.e. a seroconversion rate of 34.9% (Table 1). Our sample of free-living buffalo consisted of 96 female buffalo between the ages of 2 and 14. We measured a RVF seroprevalence rate of 39.6% (38/96) in the free-ranging population. Of the 38 RVF+ buffalo, only 5 were born prior to the previous outbreak of RVF recorded in the area, in 1999.

292 Clinical signs associated with RVF infection were noted during the outbreak in the
293 captive population. One adult female buffalo and one young calf died from RVF. Eight female
294 buffalo aborted (gestation period of buffalo is 11 months): two individuals aborted 10 month old
295 fetuses, three aborted 4-5 month old fetuses, one a 3-4 month old fetus, and the age of the fetus
296 was not recorded for the other two abortions.
297
298 CO-INFECTION PATTERNS
299 In the captive population, individual BTB + buffalo had a relative risk of acquiring RVF
300 that was 1.744 (CI 1.171 to 2.596) times higher than their BTB - counterparts. Whereas 56.25%
301 (n=124) of the BTB+ adult female buffalo seroconverted during a natural outbreak in a buffalo
302 breeding facility, only 32.26% (n=86) of the BTB - adult female buffalo seroconverted (Figure
303 1a). In the free ranging population, BTB+ buffalo (n=10) had a relative risk of being seropositive
304 for RVF that was 2.326 (CI 0.89 to 6.056) times higher than their BTB - counterparts (n=32)
305 (Fisher exact test, p=0.03) (Figure 1a). Neither age, body condition nor GI parasite egg counts
306 correlated with RVF serostatus, nor altered the direction and magnitude of the correlation
307 between RVF serostatus and BTB infection (Table 2).
308 In the captive population, buffalo with BTB were more likely to abort due to RVF than
309 those without BTB ($K=50.36$, $p<0.00001$, Figure 1b; pairwise comparisons: coinfected vs. RVF
310 only $p<0.001$, coinfected vs. BTB only $p<0.001$, coinfected vs. uninfected $p<0.001$, no other
311 significant pairwise differences), while buffalo without RVF did not suffer any abortions. While
312 7% (2/29) of the pregnant buffalo infected with only RVF aborted, 46% (6/14) of the coinfected
313 animals aborted, so the relative risk of abortion was 6.57 times greater in co-infected individuals
314 than those infected only with RVF. No buffalo infected with only BTB aborted. In previous

years there was no difference between abortion rates in the BTB+ and BTB – individuals (unpublished data).

IMMUNE MECHANISMS

Animals with BTB had significantly lower bactericidal ability of whole blood, a proxy for innate immune function, compared to those without BTB (Figure 2a, est=-0.52, SE=0.24p=0.03)). This difference was robust to accounting for animal body condition (GLM, est=-0.41, SE=0.2, p=0.4) and age (est=0.02, SE=0.04, p=0.49). We also investigated whether there was any evidence that buffalo infected with BTB had altered immune profiles that could worsen the fitness consequences of RVF infection. Buffalo with BTB mounted stronger IL12 responses to an *in vitro* stimulus with a non-specific mitogen (pokeweed) than those without BTB (Figure 2b, table 2) and a marginally stronger IL12 response to *in vitro* challenge with Rift Valley fever live viral particles (Figure 2b; two-tailed t-test, t=1.54, p=0.14).

MATHEMATICAL MODEL

We used a mathematical model to determine whether these individual changes in the likelihood of acquiring RVF could affect RVF epidemics at the herd level. We varied two key parameters: the additional RVF transmission factor for mosquitoes to BTB+ buffalo and the prevalence of BTB in the herd. We varied the increased risk of RVF infection for BTB+ animals, χ_{TB}, from 1.0-4.4. An increase in transmission from infected mosquitoes to BTB+ buffalo of χ_{TB} = 3.4 best represented the approximately 2 times greater RVF prevalence in BTB+ buffalo observed in our free-ranging and captive populations. This value depends on the assumed BTB

prevalence and whether there is immunity to RVF from previous exposure. We varied BTB prevalence, $ø_{TB}$, from 0 to 1.

In agreement with the outbreak data, the model predicts higher RVF seroprevalence in BTB + buffalo than in BTB - at the end of the outbreak. However, we found that increasing BTB prevalence within a herd increased both the overall magnitude of an RVF outbreak and the RVF seroprevalence in BTB- individuals (Figure 3). This implies that the presence of BTB increases RVF infection risk for all members of the herd, not just those infected with BTB. Outbreak size responded nonlinearly to increased BTB prevalence at a fixed transmission factor with outbreak size increasing more rapidly as BTB prevalence increased (Figure 3). The relative effect of BTB prevalence and the transmission factor on RVF dynamics (time to peak and length of outbreak) varied across the parameter ranges explored (Figure 4).

Discussion:

Our results suggest that an emerging pathogen, such as BTB, may not only have direct effects on the host, but also indirect effects by altering the infection patterns of diseases previously existing within the host population. Buffalo in both the free-ranging and captive populations were approximately twice as likely to acquire RVF when previously infected with BTB, providing strong evidence that BTB affects host susceptibility to other pathogens. Because these patterns were duplicated in two independent populations, we investigated possible mechanisms behind the correlations.

BTB in cattle causes dynamic alterations to the host immune response over time [46], whereby animals may have reduced ability to mount immune responses to protect them from micro parasites such as RVF [47] . Pirson et al [48] suggested that receptors and function of antigen presenting cells were suppressed in BTB infection, which would decrease the host's

ability to respond to an insult from a pathogen. Concordant with these findings we saw that animals with BTB had suppressed innate immune responses, as measured by the bactericidal ability of whole blood. This reduced ability to respond to a pathogen with a strong innate response may increase the likelihood that animals with BTB become infected with other pathogens that require suppression by the innate immune system, such as most acute viral pathogens.

We used a mathematical model to show that changes in host susceptibility to RVF due to BTB infection in individual buffalo could increase the intensity of RVF epidemics in the entire herd. As the prevalence of BTB increased, the size of RVF epidemics in buffalo increased, with more disease occurring in both BTB+ and BTB- buffalo. The response of RVF outbreak size to BTB prevalence was nonlinear, with outbreak size increasing more rapidly at higher BTB prevalence, indicating a complex relationship between RVF population level dynamics and coinfection (Figure 3 and Appendix 1). At BTB prevalence above 20%, with a transmission factor increase of 3.4 (which best represented our data from the free-living and captive populations), BTB significantly alters the spread of RVF. At medium BTB prevalence (40-50%), like we see in Southern KNP, with a transmission factor increase of 3.4 the size of an RVF outbreak in buffalo more than doubles. In addition, the presence of BTB changed the shape of the epidemic curve depending on the transmission factor and BTB prevalence (Figure 4). At BTB prevalence of 40-50%, with a transmission factor increase of 3.4 increases the time to peak, and overall length of the outbreak. Within Kruger National Park, BTB prevalence ranges from 0-1% in the northern section of the park to 50% in the southern section of the park where BTB first was found [49]. As BTB continues to move north within KNP, crossing into Zimbabwe [50], RVF epidemics in African buffalo may increase in size. Whether this potential increase in

383 RVF-infected buffalo will increase the risk of outbreaks extending into humans, domestic
384 livestock, or free-ranging ruminants needs to be investigated.

385 Animals with BTB had greatly increased rates of abortion due to RVF, with abortions an
386 estimated 6 times higher in the BTB+ individuals than the BTB- individuals. While BTB alone
387 may have only minor population level effects on buffalo [51,52], it may exacerbate the effects of
388 other diseases such as RVF, which could therefore influence the impact on host population
389 dynamics. Future work should focus on understanding whether the alteration of individual level
390 buffalo-RVF interactions scales up to affect buffalo population dynamics.

391 The increase in RVF abortion in animals previously infected with BTB may be due to an
392 immune mediated interaction between BTB and RVF. We investigated this idea by comparing
393 the production by BTB + and BTB - buffalo, of a pro-inflammatory cytokine (IL12) in response
394 to *in vitro* challenge with RVF virus or a generic stimulant, pokeweed. IL12 is produced in
395 response to micro parasite infection, as part of a cell-mediated or T-helper cell type 1 (Th1) –
396 mediated response [53]. IL12 is a key cytokine involved in ramping up the inflammatory
397 response that allows intracellular micro parasites to be eliminated from the host's body. While
398 inflammation is an important component of the animal's repertoire of anti-micro parasite
399 defenses, it also incurs substantial costs in form of collateral damage, or immunopathology. For
400 example, Thacker et al [22] found that BTB-infected cows with systemically increased Th1
401 cytokine mRNA expression had increased pathology associated with BTB infection. Studies with
402 other pathogens have also found that cows with a proinflammatory (Th1) skew to their immune
403 systems suffered increased abortions [54,55]. In our study, buffalo with BTB produced more
404 IL12 than uninfected buffalo, in response to *in vitro* stimulation with Rift Valley fever vaccine
405 and pokeweed. This suggests BTB infection modifies buffalo immunity toward a Th1 or pro-

406 inflammatory skew, similar to previous observations in cows with BTB [22]. Once infection has
407 occurred, this skew towards a Th1 immune response may help eliminate the pathogen more
408 quickly but may come at a great cost to the individual - increasing the likelihood of abortion or
409 other clinical signs in coinfected individuals. Mechanistic work including experimental
410 infections will be needed to clarify whether the observed pro-inflammatory skew in BTB +
411 buffalo is indeed causal of exacerbated fitness consequences during co-infection with RVF.
412 It is also possible that other mechanisms besides immunity may be important in
413 driving the patterns noted here. For example, the patterns could be resource-mediated, but
414 this seems unlikely since the parasites do not utilize the same resources in the host. Other
415 members of the parasite could also play a role. Future work will need to investigate parasite
416 and pathogen communities beyond 2-way interactions and evaluate whether the altered immune
417 dynamics are the primary mechanism for increased susceptibility to RVF in BTB+ individuals
418 and to what extent other mechanisms may play a role.
419 In conclusion, we found that buffalo previously infected with BTB had increased risk of
420 acquiring RVF, and also had an increased risk of aborting due to RVF. BTB also magnified the
421 intensity of RVF outbreaks in a mathematical model, which has implications for spillover of this
422 zoonotic infection to livestock and people. Our study points to a new frontier – understanding
423 how emerging pathogens modify disease dynamics and health outcomes of previously
424 established infections. If new enemies expose the pathogenic potential of old diseases, emerging
425 infections may pose more significant risks for population health than anticipated.
426
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Table 1: Age and sex patterns of RVF seroconversion of captive buffalo during a natural outbreak in 2008.

	Number seroconverted	Total number tested	Percent Seroconverted
Adult Cows	40	124	32.26%
Adult Bulls	3	19	15.79%
Calves under 1 year	21	26	80.77%

Table 2: A generalized linear model (binomial distribution, log link, df=92) was performed to further evaluate the correlation between BTB status and RVF seropositivity in free-ranging African buffalo. Age, pregnancy status, overall body condition, fecal nematodes and coccida did not alter the positive association between BTB and RVF.

	Estimate	**SE**	**p value**
Age	0.20	0.12	0.105
BTB Status (Positive)*	1.51	0.76	0.046*
Pregnancy Status (Yes)	-0.21	0.77	0.785
Body Condition	0.11	0.37	0.768
Nematodes eggs per gram	0.001	0.001	0.429
Coccidia oocysts per gram	-0.001	0.004	0.660

Table 3: Animals with BTB had stronger IL12 response to pokeweed even after accounting for animal body condition, year of capture and baseline IL12. The table contains estimates, SE and p values for the model parameters in a generalized linear model (Gaussian family, log link) with formula (log IL12 Difference~IL12 Plate + IL12 Base circulating level + Animal Body Condition + BTB Status with Animal ID and IL12 Plate Number as random effects).

	Estimate	**SE**	**p value**
Circulating IL12	-0.003	0.0002	<0.01*
Capture Year	-0.001	0.001	0.56
Animal Body Condition	-0.0003	0.0008	0.72
BTB Status (+)	0.002	0.008	0.04*

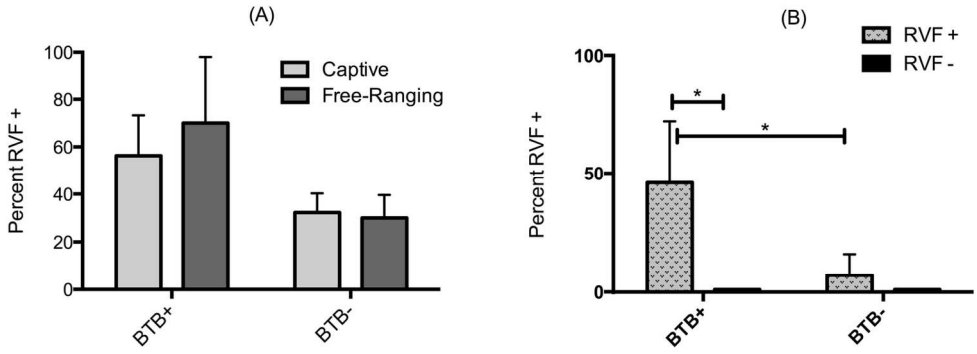

Figure 1 - Effect of BTB on RVF Incidence (A) and Abortion (B). (A) BTB + buffalo were more likely to acquire RVF infection (Fisher exact test, p=0.0147) during an outbreak in the captive herd (panel A, light grey) and are more likely to be seropositive (Fisher exact test, p=0.03) in a free-ranging herd (panel A, dark grey). Animals with BTB were more likely to abort from RVF, than those without BTB (panel B). No animals without RVF aborted; a line was placed just above 0 on the y-axis for visibility. Stars represent significant differences on a Kruskal Wallis ANOVA with Dunn pairwise comparisons).
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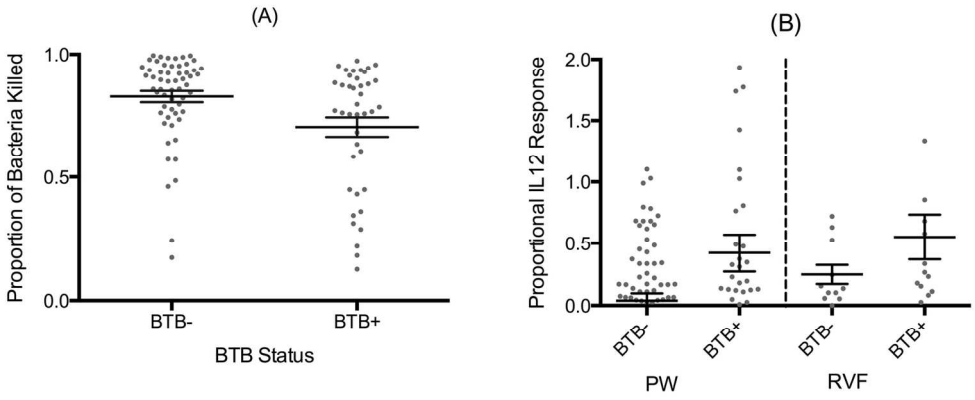

Figure 2 - Immunologic Effects of BTB. Animals with BTB had reduced bactericidal ability of whole blood (A) and increased IL12 response (B). Each point is an individual animal's proportion of bacteria killed (A) or IL12 response to mitogen (B) with the mean and SEM represented by the line and error bars respectively.
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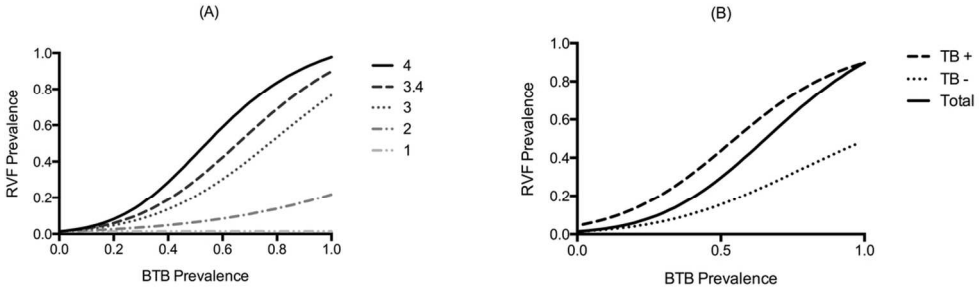

Figure 3 - Effect of BTB on RVF epidemic size. Panel A shows that as BTB prevalence increases, so does the total RVF outbreak size. The extent of the increase depends on the factor by which transmission is increased due to BTB (transmission factors 1, 2, 3 and 3.4 and 4 are shown in the figure). A transmission factor increase of 3.4 best represented the data from the captive and free-living herds. (B) When the transmission factor for BTB+ buffalo was fixed at 3.4 times the rate in BTB- buffalo, increasing BTB prevalence resulted in increased predicted RVF prevalence for both BTB+ and BTB- buffalo. At BTB prevalence of 40-50%, as seen in Southern KNP, the RVF outbreak is predicted to be more than twice as large as in herds without BTB.
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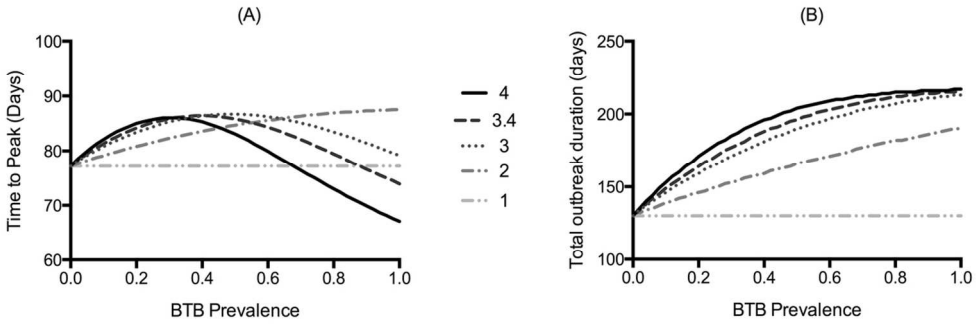

Figure 4: BTB prevalence and the relative increase in risk of RVF transmission for BTB+ buffalo, χ_{TB}, changed the shape of the epidemic curve for RVF. Higher BTB prevalence changed both the time to peak RVF prevalence (A) and the total duration of the outbreak (B). At low BTB prevalence, increasing BTB prevalence results in a longer time to peak RVF prevalence. However at high BTB prevalence, increasing BTB prevalence results in a faster outbreak and a shorter time to peak RVF prevalence.
55x19mm (600 x 600 DPI)